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(54) Novel, distinct family of human leukocyte interferons, compositions containing them, methods for their production, and DNA and transfected hosts therefor.

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Description

Field of the Invention

The present invention relates to a novel, distinct family of human leukocyte interferon proteins (designated herein by HuIFN-αII or HuIFN-α_C and to species thereof as HuIFN-αII.1 or HuIFN-α_C1, and so forth) which are useful in the treatment of viral and neoplastic diseases, and to the means for producing such interferon proteins. In general, the present invention finds its basis in the field of recombinant DNA technology which has been employed to discover and produce this novel, distinct family of human leukocyte interferons.

The publications and other materials hereof used to illuminate the background of the invention, and in particular cases, to provide additional details respecting its practice are hereby incorporated by reference, and for convenience are numerically referenced by the following text and respectively grouped in the appended bibliography.

Background of the Invention

A human leukocyte interferon was first discovered and prepared in the form of very crude precipitates from natural source (1). Following this work was the substantial discovery that human leukocyte interferons exist as a class or family of proteins all exhibiting close homology and varying degrees of similar in kind antiviral activity. This work has been documented in several references as follows: (2, 3, 4, 5). This family of leukocyte interferons (commonly referred to in abbreviated form as HuIFN-α) has been reported to be composed of upwards of 15 or more individual species, having varying degrees of similar in kind antiviral activity. Characteristically, these human leukocyte interferon species have been identified by amino acid sequences consisting of from about 165 to 166 amino acids in their mature forms, by the underlying DNA sequence for each and by identification of glycosolation sites and reported antiviral activity in animal species. Indeed, at least one of these human leukocyte interferon proteins is enjoying success in certified human clinical studies. These interferon species have been and are being produced via recombinant DNA technology, notably employing the workhorse in this field, namely a transfected *E. coli* microorganism. Thus, these previous discoveries have enabled the production currently of sufficient quantities of human leukocyte interferon species via recombinant DNA technology from a transfected host system as to permit the recovery of relatively large amounts of very pure protein finding use for requisite clinical studies. These achievements have been reported in the references cited previously as well as other references forming a part of the state of the art currently.

As a result of the extensive studies that various workers have expended on the study of the human leukocyte interferon family, it was thought beyond doubt that the human leukocyte interferons that have been discovered and studied were composed within a single family of proteins sharing characteristics of homology, amino acid length and antiviral activity. Similar research attended corresponding success for animal, notably bovine, interferons (6).

A second class of human interferons is represented by the so called human fibroblast interferon (β-interferon, or HuIFN-β). Although extensive research into this compound has been conducted, surprisingly it is thought to be a single polypeptide or protein, in contradistinction to the leukocyte series where, as noted above, upwards of 15 or more species are thought to exist within the general definitional term of human leukocyte interferon (7).

A third class of human interferons is represented by human gamma interferon (HuIFN-γ) (8, 9). Although human gamma interferon has been reported to exhibit the antiviral and antiproliferative properties characteristic of the human interferons in the leukocyte and fibroblast series, its properties are distinct in that, in contrast to the leukocyte and beta interferons, it is of shorter amino acid length and is pH 2 labile (10). Because of these distinctions, human gamma interferon is thought to be slated more for indications of antiproliferative activity with indications of substantial use in the treatment of cancer patients. Central and independent research has therefore attended the human gamma interferon molecule.

Inasmuch as human fibroblast interferon (HuIFN-β) and human leukocyte interferons (HuIFN-α) are similar structurally (i.e. amino acid length and homologous sequence) and biologically (i.e. antiviral activity), it was thought by many researchers odd that the human leukocyte interferon would be composed of a family of multiple species whereas in the human fibroblast interferon case only one gene has so far been located, indicating evolutionary divergence and expansion to multiple genes within the leukocyte family but retention of a single distinct gene in the case of fibroblast interferon.

Because of this curiosity, the inventors of the present invention chose to search for additional HuIFN-β genes. This effort was manifested by screening at low hybridization stringency a human genomic DNA library (11) utilizing a DNA probe prepared from a fragment spanning the mature coding region of the known HuIFN-β gene. This research resulted in the surprising, serendipitous discovery of a novel, distinct family of human leukocyte interferons not previously known or thought to exist. This discovery of a novel, distinct family of human leukocyte interferons forms the basis of the present invention.

Summary of the Invention

The present invention relates to the discovery of a novel and distinct family or group within the human leukocyte interferon class of compounds. This new family or group of human leukocyte interferons,

distantly related gene which only hybridized to the HuIFN-β probe at low stringency. A 4.1 kilobase *Hind*III fragment containing the hybridizing region from one of these clones, λ24.1, was subcloned into pBR322 for further characterization by restriction endonuclease mapping (Figure 4) and nucleotide sequencing.

DNA sequence analysis of this fragment (Figure 6) reveals an interferon gene exhibiting substantially more nucleotide homology in its coding region with the HuIFN-α genes (for example, 70 percent with HuIFN-α_1, Table 1) than with HuIFN-β (48 percent). Similarly, while the HuIFN-α_1 protein and the gene product of λα24.1 each share only 30 percent amino acid homology with HuIFN-β, they are approximately 58 percent homologous with one another. It was concluded that the gene contained on λα24.1 encodes an IFN-α rather than an IFN-β. The sequence of this protein, however, is surprisingly dissimilar to the other HuIFN-α gene products.

The novel HuIFN-α contains five or six additional amino acids at its carboxy terminus, three of which are identical in each protein (Figure 6). These similarities are reflected at the level of nucleotide homology as well (Table 1). Taken together, these observations strongly suggest that this novel HuIFN-α represents a homologous gene product, distinct from the class I IFN-α gene family which includes the previously sequenced functional HuIFN-α genes. Accordingly, we have named this gene HuIFN-$\alpha_{II}1$ to distinguish it from the class I HuIFN-α genes.

The HuIFN-$\alpha_{II}1$ sequence contains a potential glycosylation sequence, asn-met-thr, at positions 78—80. Interestingly, a similar sequence is found at the same position in HuIFN-β which is known to be modified *in vivo* by carbohydrate addition (20).

To examine the possibility that HuIFN-$\alpha_{II}1$ might define a new family of IFN-α genes in the human genome, blot-hybridization analysis was performed with human genomic DNA utilizing probes derived from the HuIFN-α_1 and HuIFN-$\alpha_{II}1$ coding region under stringent conditions which do not permit cross-hybridization of the two genes. The results of such an experiment, shown in Figure 5, indicate that the HuIFN-α_1 and HuIFN-$\alpha_{II}1$ probes define distinct gene families. The HuIFN-$\alpha_{II}1$ probe demonstrates the presence of 6—7 class II genes in the human genome.

Expression of class II IFN-α genes is inducible by virus:

The HuIFN-$\alpha_{II}1$ protein or related class II IFN-α gene products have not been identified in interferon preparations from virally-induced cell lines (21, 22), nor have the corresponding DNA sequences been found in cDNA libraries prepared from lymphoblastoid cell lines (3) or peripheral blood lymphocytes induced by viruses. To determine whether class II HuIFN-α genes are transcribed in response to virus infection, RNA from the peripheral blood lymphocytes of two donors induced with either Sendai or Newcastle Disease virus was analyzed by blot hybridization. Following a six hour incubation with virus, polyA+RNA was isolated from the cultures, electrophoresed on formaldehyde gels, transferred to nitrocellulose filters and hybridized with either a class I (HuIFN-α_2) or class II (HuIFN-$\alpha_{II}1$) probe. As seen in Figure 7, transcription of both the class I (Fig. 7A) and class II (Fig. 7B) IFN-α genes is induced by both Newcastle Disease virus (lanes 2 and 5) and Sendai virus (lanes 3 and 6), and is not detectable in uninduced cultures from either donor (lanes 1 and 4). To compare the levels of expression of the class I and class II genes, filters hybridized with each probe were exposed to film until DNA markers containing the coding regions of either HuIFN-α_2 (Figure 7A) or HuIFN-$\alpha_{II}1$ (Figure 7B) gave signals of equal intensity (data not shown). From this analysis it appears that the class II genes are transcribed at a level comparable to the class I genes (Figure 7). In addition, it appears that Sendai virus induces several-fold more class I and class II interferon message than NDV. This suggests that the class I and class II genes are regulated similarly in response to viral infection.

To confirm the conclusion that the IFN-$\alpha_{II}1$ gene is expressed, a complementary DNA library was constructed from polyA(+) RNA isolated from Sendai-induced peripheral blood lymphocytes. A HuIFN-$\alpha_{II}1$ coding region probe was employed to screen 10,000 plaques under stringent hybridization conditions. Two HuIFN-$\alpha_{II}1$ clones were recovered. DNA sequence analysis showed that the longer of the two cDNA clones extended from the poly(A) of the mRNA to within the sequence encoding the signal peptide of HuIFN-$\alpha_{II}1$. The corresponding sequence within the HuIFN-$\alpha_{II}1$ gene is indicated in Figure 8.

Class II IFN-α genes encode proteins with antiviral activity:

To determine whether class II IFN-α genes encode active proteins and to compare their host range with those of class I IFN-α polypeptides, bacterial vectors were constructed for the expression of HuIFN-$\alpha_{II}1$ genes. The resulting plasmids join a *trp* operon promoter, ribosome-binding site and initiator methionine codon to the first amino acid residue of each mature IFN-α coding region. As shown in Table 2, extracts prepared from *E. coli* strains transformed with each of the three plasmids and grown under conditions leading to depletion of tryptophan from the growth media contain significant amounts of antiviral activity as measured by cytopathic effect inhibition assays. The relative activities of each IFN-α were compared with those of two class I HuIFN-α proteins, HuIFN-α_2 and HuIFN-α_1, on a bovine cell line (MDBK) cell line challenged with vesicular stomatitis virus and on a human lung carcinoma (A549) cell line challenged with encephalomyocarditis virus. HuIFN-$\alpha_{II}1$ and HuIFN-α_2, show approximately equal activity on each cell type. The class II IFN-α proteins therefore appear to have specificities which overlap those of several class I IFN-α gene products in these as well as other cell lines.

To further characterize the antiviral activity associated with HuIFN-$\alpha_{II}1$, we examined the ability of

antisera prepared against IFN-α and IFN-β to neutralize HuIFN-$\alpha_{II}1$ activity. Anti-HuIFN-β did not significantly effect the activity of HuIFN-$\alpha_{II}1$, while an antisera prepared against Sendai-virus induced interferon from human leukocyte cultures did neutralize HuIFN-$\alpha_{II}1$ antiviral activity (Table 3). Since the latter induction protocol has been shown to result primarily in the production of HuIFN-α rather than HuIFN-β, these results confirm the assignment of HuIFN-$\alpha_{II}1$ to the IFN-α family, made on the basis of protein homology.

Two distinct IFN-α gene families:

Previous studies have described a family of approximately fifteen non-allelic human IFN-α genes, which are related by at least 85 percent nucleotide homology in their coding regions (3). For clarity, these genes are referred to as the HuIFN-α_I family. Each gene encodes a functional interferon polypeptide as determined by its ability to program the expression of antiviral activity in *E. coli*.

Such a gene was identified, HuIFN-$\alpha_{II}1$, among a collection of clones initially isolated by screening a human genomic library with a HuIFN-β cDNA probe. Comparison of DNA homologies, however, as well as antigenicity studies on the HuIFN-$\alpha_{II}1$ protein expressed in *E. coli*, firmly establish that this gene encodes an IFN-α rather than an IFN-β protein. HuIFN-$\alpha_{II}1$ encodes a mature polypeptide of 172 amino acid residues. These results demonstrate the existence of two homologous, but distinct IFN-α gene families in the human genome.

Southern blot analysis of human DNA suggests that the class II HuIFN-α gene family may contain as many as 6—7 different members.

The members of the class I HuIFN-α family, as well as HuIFN-β, have been localized to chromosome 9, (23), while the gene for HuIFN-γ is found on chromosome 12 (24). Recent experiments indicate that most, if not all, members of the class II HuIFN-α family are also located on chromosome 9.

Transcriptional control of class II IFN-α genes:

Differential regulation of IFN-α mRNA levels has been suggested by the greater than ten-fold range in frequency with which individual HuIFN-α mRNA sequences are found in virally-induced cell cultures. In our efforts to compare transcriptional regulation of class I and II IFN-α genes, we have employed Newcastle Disease virus (NDV) or Sendai virus to induce interferon synthesis in human peripheral blood lymphocytes. Comparable levels of class I and class II mRNA were readily observed in polyA(+)-RNA prepared from each culture following six hours of viral induction (Figure 7). In addition, Sendai virus appeared to induce several-fold more IFN-α transcripts of class II, as well as class I types, indicating similar transcriptional activation of either IFN-α family by viral induction. In light of the observation that class II cDNA clones were not represented among libraries prepared from Sendai virus-induced cultures of human leukocytes or a human myeloblastoid cell line, this is the first evidence for the viral induction of class II IFN-α synthesis. This result has been further confirmed by the isolation of a complementary DNA clone which includes the 3'-end of the IFN-$\alpha_{II}1$ mRNA, and most of its coding region (Figure 6).

Hybridization conditions and probes:

Hybridizations were performed in 5×SSC (1×SSC is 0.15 M NaCl, 0.015 M sodium citrate), 5× Denhardt's solution (12), 0.1 percent sodium dodecyl sulfate (SDS), 0.1 percent sodium pyrophosphate, 50 µg/ml sonicated, denatured salmon sperm DNA and 10 percent sodium dextran sulfate, containing either 20 percent or 50 percent formamide for non-stringent and stringent conditions, respectively. After incubation at 42°C, filters were washed at room temperature in 2×SSC, 0.2 percent SDS (non-stringent) or 42°C in 0.2×SSC, 0.1 percent SDS (stringent). ^{32}P-labelled probes were prepared as described by (13). For the analysis of class I and II genes in human DNA at high stringency (Figure 5), the following fragment was employed (each contained the mature coding region of the corresponding IFN-α gene): class I human, the 565 bp *Eco*RI fragment of pHuIFN-α_2*21 Bgl*II hybrid (14); class II human, the 990 bp *Xba*I-*Acc*I fragment of pHuIFN-$\alpha_{II}1$ (see Figure 8 for description of plasmids).

Construction and screening of phage libraries:

The HuIFN-$\alpha_{II}1$ gene was isolated from a human fetal liver/bacteriophage λ Charon 4A library constructed by Lawn *et al.* (11) utilizing a probe prepared from the 501 bp *Xba*I-*Bgl*II fragment encoding the mature HuIFN-β protein.

DNA Sequence analysis:

DNA sequences were determined as described by (18) or by subcloning DNA fragments into M13 mp8 and mp9 vectors (17) and employing the dideoxy chain termination method (18).

Preparation and analysis of virally induced RNA:

Peripheral blood lymphocytes (2×10^8) were resuspended at 4×10^6 cells per ml in RPMI 1640 containing 5 percent (heat inactivated) fetal calf serum. Cultures were incubated in T-175 flasks (Falcon) and induced cultures were treated with 25 UAU/10^6 cells of Newcastle Disease virus or Sendai virus. Following six hours of incubation with virus, the cultures were treated with 0.05 percent EDTA and cells were harvested by centrifugation, washed once with ice-cold medium, and polyA(+) RNA was prepared (18). Formaldehyde

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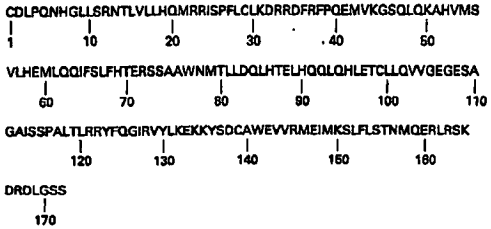
21. Zoon *et al., Science 207,* 527 (1980).
22. Allen *et al., Nature 287,* 408 (1980).
23. Owerbach *et al., Proc. Natl. Acad. Sci. (USA) 78,* 3123 (1981).
24. Naylor *et al., J. Exp. Med. 57,* 1020 (1983).
25. Huynh *et al., DNA Cloning Techniques: A Practical Approach,* Ed. D. Glover, IRL Press (1984).

Claims for the Contracting States: BE CH DE FR GB IT LI LU NL SE

1. A genomic or complementary DNA sequence encoding a human leukocyte interferon which
(i) consists of a mature polypeptide of about 172 amino acids;
(ii) exhibits about 70% homology at the DNA level with IFN-α1 of class I;
(iii) exhibits about 48% homology at the DNA level with human IFN-β and
(iv) which hybridises with the 501 bp XbaI-BglII restriction fragment of human genomic DNA encoding the mature HuIFN-β protein under conditions of low stringency represented by hybridisation in 6×SSC, 5× Denhardt's solution, 0.1% SDS, 0.1% sodium pyrophosphate, 50 µg/ml sonicated denatured salmon sperm DNA and 10% sodium dextran sulfate, containing 20% formamide, incubated at 42°C and washed at room temperature in 2×SSC and 0.2% SDS.

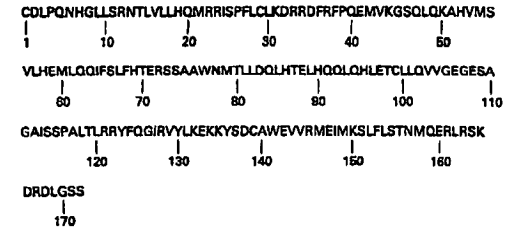
2. A DNA sequence as claimed in claim 1 encoding a human leukocyte interferon of the following amino acid sequence:



3. A DNA sequence according to claim 1 encoding an allele or a functional derivative of a human leukocyte interferon the sequence of which is defined in claim 2.

4. A human leukocyte interferon in mature form the amino acid sequence of which is encoded by the DNA of claim 1.

5. A human leukocyte interferon of class all.1 having the following amino acid sequence:



6. A physiologically functional human leukocyte interferon which is an allele or a derivative of the compound of claim 5.

7. A pharmaceutical composition comprising a human leukocyte interferon of any one of claims 4 to 6 and a pharmaceutically acceptable carrier.

8. A human leukocyte interferon of any one of claims 4 to 6 for pharmaceutical use.

9. The use of a human leukocyte interferon of any one of claims 4 to 6 in the manufacture of an anti-viral medicament.

10. An expression vector containing a DNA sequence coding for a human leukocyte interferon according to any one of claims 4—6 and capable of expressing said human leukocyte interferon.

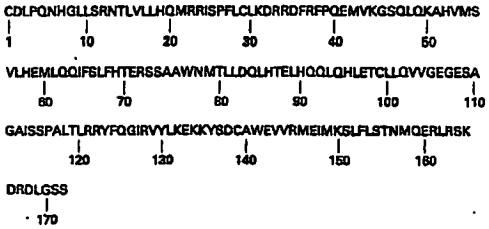
11. A microorganism or cell culture transfected with an expression vector as claimed in claim 10.

12. A process for producing a human leukocyte interferon as claimed in any one of claims 4—6, which process comprises expressing said interferon in a recombinant host organism transfected with an expression vector as claimed in claim 10.

Claims for the Contracting State: AT

1. A process which comprises the preparation of a genomic or complementary DNA sequence encoding a human leukocyte interferon which
(i) consists of a mature polypeptide of about 172 amino acids;
(ii) exhibits about 70% homology at the DNA level with IFN-α1 of class I;
(iii) exhibits about 48% homology at the DNA level with human IFN-β and
(iv) which hybridises with the 501 bp XbaI-BglII restriction fragment of human genomic DNA encoding the mature HuIFN-β protein under conditions of low stringency represented by hybridisation in 6×SSC, 5× Denhardt's solution, 0.1% SDS, 0.1% sodium pyrophosphate, 50 µg/ml sonicated denatured salmon sperm DNA and 10% sodium dextran sulfate, containing 20% formamide, incubated at 42°C and washed at room temperature in 2×SSC and 0.2% SDS.

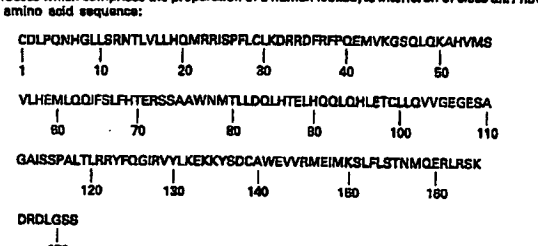
2. A process according to claim 1 in which the human leukocyte interferon has the following amino acid sequence:



3. A process according to claim 1 wherein the DNA encodes an allele or a functional derivative of a human leukocyte interferon the sequence of which is defined in claim 2.

4. A process which comprises the preparation of a human leukocyte interferon in mature form the amino acid sequence of which is encoded by the DNA of claim 1.

5. A process which comprises the preparation of a human leukocyte interferon of class all.1 having the following amino acid sequence:



6. A process which comprises the preparation of a physiologically functional human leukocyte interferon which is an allele or a derivative of the compound of claim 5.

7. The use of a human leukocyte interferon of any one of claims 4 to 6 in the production of a pharmaceutical preparation.

6. Verfahren, das die Herstellung eines physiologisch funktionellen Human-Leukozyt-Interferons umfaßt, das ein Allel oder ein Derivat der Verbindung nach Anspruch 5 ist.

7. Verwendung eines Human-Leukozyt-Interferons nach einem der Ansprüche 4 bis 6 für die Herstellung eines pharmazeutischen Präparates.

8. Verwendung eines Human-Leukozyt-Interferons nach einem der Ansprüche 4 bis 6 für die Herstellung eines Antivirus-Arzneimittels.

9. Verfahren, das die Herstellung eines Expressionsvektors umfaßt, der eine DNA Sequenz enthält, die für ein Human-Leukozyt-Interferon nach einem der Ansprüche 4 bis 6 kodiert und fähig ist, das genannte Human-Leukozyt-Interferon zu exprimieren.

10. Mikroorganismus oder Zellkultur, der bzw. die mit einem Expressionsvektor nach Anspruch 9 transfiziert ist.

11. Verfahren zur Herstellung eines Human-Leukozyt-Interferons nach einem der Ansprüche 4 bis 6, welches Verfahren das Exprimieren des genannten Interferons in einem rekombinanten Wirtsorganismus umfaßt, der mit einem Expressionsvektor nach Anspruch 10 transfiziert ist.

Revendications pour les États Contractants: BE CH DE FR GB IT LI LU NL SE

1. Séquence d'ADN génomiques ou complémentaires codant un interféron de leucocytes humains qui
(i) consiste en un polypeptide mûr d'environ 172 acides aminés;
(ii) présente une homologie d'environ 70% au niveau d'ADN avec IFN-α1 de la classe I;
(iii) présente une homologie d'environ 48% au niveau d'ADN avec IFN-β humain et
(iv) qui s'hybride avec le fragment de restriction XbaI-BglII de 501 pb de l'ADN génomique humain codant la protéine mûre de HuIFN-β dans des conditions de faible rigueur représentées par une hybridation dans 5×SSC, 5× solution de Denhardt, 0,1% SDS, 0,1% pyrophosphate de sodium, 50 µg/ml d'ADN de sperme de saumon dénaturé et soniqué et 10% de sulfate de dextrane sodium, contenant 20% de formamide, avec incubation à 42°C et lavage à température ambiante dans 2×SSC et 0,2% SDS.

2. Séquence d'ADN selon la revendication 1 codant un interféron de leucocytes humains de la séquence d'acides aminés qui suit:

```
CDLPQNHGLLSRNTLVLLHQMRRISPFLCLKDRRDFRFPQEMVKGSQLQKAHVMS
|         |         |         |         |         |
1         10        20        30        40        50

VLHEMLQQIFSLFHTERSSAAWNMTLLDQLHTELHQQLQHLETCLLQVVGEGESA
|         |         |         |         |         |         |
60        70        80        90        100       110

GAISSPALTLRRYFQGIRVYLKEKKYSDCAWEVVRMEIMKSLFLSTNMQERLRSK
          |         |         |         |         |
          120       130       140       150       160

DRDLGSS
|
170
```

3. Séquence d'ADN selon la revendication 1 codant un allèle ou un dérivé fonctionnel d'un interféron de leucocytes humains dont la séquence est définie à la revendication 2.

4. Interféron de leucocytes humains sous forme mûre dont la séquence d'acides aminés est codée par l'ADN de la revendication 1.

5. Interféron de leucocytes humains de la classe αI.1 ayant la séquence d'acides aminés qui suit:

```
CDLPQNHGLLSRNTLVLLHQMRRISPFLCLKDRRDFRFPQEMVKGSQLQKAHVMS
|         |         |         |         |         |
1         10        20        30        40        50

VLHEMLQQIFSLFHTERSSAAWNMTLLDQLHTELHQQLQHLETCLLQVVGEGESA
|         |         |         |         |         |         |
60        70        80        90        100       110

GAISSPALTLRRYFQGIRVYLKEKKYSDCAWEVVRMEIMKSLFLSTNMQERLRSK
          |         |         |         |         |
          120       130       140       150       160

DRDLGSS
|
170
```


6. Interféron de leucocytes humains physiologiquement fonctionnel qui est un allèle ou un dérivé du composé de la revendication 5.

7. Composé pharmaceutique comprenant un interféron de leucocytes humains selon l'une des revendication 4 à 6 et son véhicule acceptable en pharmacie.

8. Interféron de leucocytes humains selon l'une des revendication 4 à 6 pour un usage pharmaceutique.

9. Utilisation d'un interféron de leucocytes humains selon l'une des revendications 4 à 6 dans la fabrication d'un médicament antiviral.

10. Vecteur d'expression contenant une séquence d'ADN codant pour un interféron de leucocytes humains selon l'une des revendications 4—6 et capable d'exprimer ledit interféron de leucocytes humains.

11. Micro-organisme ou culture de cellules transfecté au moyen d'un vecteur d'expression selon la revendication 10.

12. Procédé de production d'un interféron de leucocytes humains selon l'une quelconque des revendications 4—6, lequel procédé comprend l'expression dudit interféron dans un organisme hôte recombinant transfecté avec un vecteur d'expression selon la revendication 10.

Revendications pour l'Etant Contractant: AT

1. Procédé qui comprend la préparation d'une séquence d'ADN génomiques complémentaires codant un interféron de leucocytes humains qui
(i) consiste en un polypeptide mûr d'environ 172 acides aminés;
(ii) présente une homologie d'environ 70% au niveau d'ADN avec IFN-α 1 de la classe I;
(iii) présente une homologie d'environ 48% au niveau d'ADN avec IFN-β humain et
(iv) qui s'hybride avec le fragment de restriction XbaI-BglII de 501 pb de l'ADN génomique humain codant la protéine mûre de HuIFN-β dans des conditions de faible rigueur représentées par une hybridation dans 5×SSC, 5× solution de Denhardt, 0,1% SDS, 0,1% pyrophosphate de sodium, 5 µg/ml d'ADN de sperme de saumon dénaturé et soniqué et 10% de sulfate de dextrane sodium, contenant 20% de formamide, avec incubation à 42°C et lavage à température ambiante dans 2×SSC et 0,2% SDS.

2. Procédé selon la revendication 1 où l'interféron de leucocytes humains a la séquence d'acides aminés qui suit:

```
CDLPQNHGLLSRNTLVLLHQMRRISPFLCLKDRRDFRFPQEMVKGSQLQKAHVMS
|         |         |         |         |         |
1         10        20        30        40        50

VLHEMLQQIFSLFHTERSSAAWNMTLLDQLHTELHQQLQHLETCLLQVVGEGESA
|         |         |         |         |         |         |
60        70        80        90        100       110

GAISSPALTLRRYFQGIRVYLKEKKYSDCAWEVVRMEIMKSLFLSTNMQERLRSK
          |         |         |         |         |
          120       130       140       150       160

DRDLGSS
|
170
```

3. Procédé selon la revendication 1 où l'ADN code un allèle ou un dérivé fonctionnel d'un interféron de leucocytes humains dont la séquence est définie à la revendication 2.

4. Procédé qui comprend la préparation d'un interféron de leucocytes humains sous forme mûre dont la séquence d'acides aminés est codée par l'ADN de la revendication 1.

5. Procédé qui comprend la préparation d'un interféron de leucocytes humains de la classe αI.1 ayant la séquence d'acides aminés qui suit:

Fig.2A.

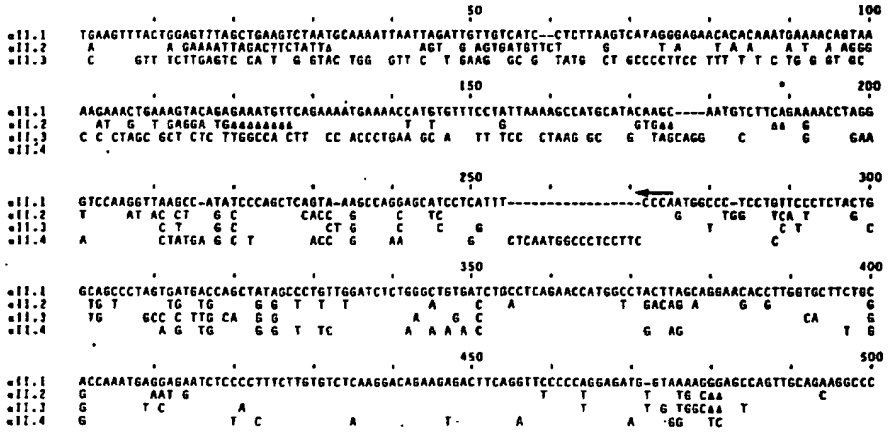
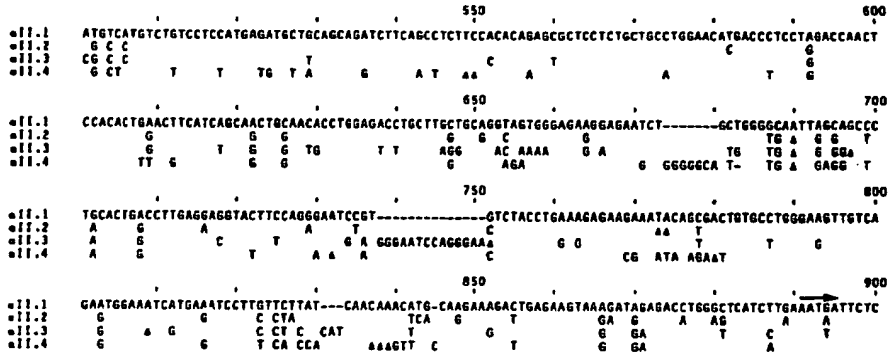


Fig.2B.



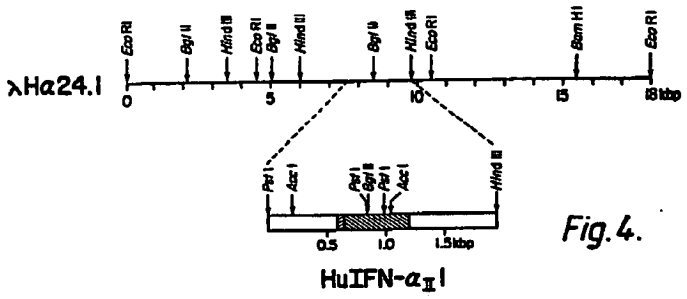


Fig.4.

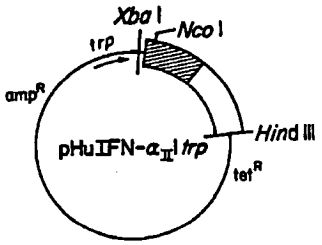


Fig.8.

Fig.5.

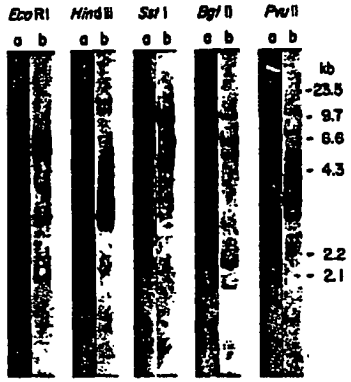


Fig.6C.

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160                   166
glu arg leu arg arg lys glu OC
GAA AGA TTA AGG AGG AAG GAA TAA TAT CTG GTC CAA CAT GAA AACAATTCTTATTGACTCATACACCAGGTCACGCTTTCATGAATTCTGTC      ATTTCAAA
GAA AGA CTG AGA AGT AAA GAT AGA GAC CTG GGC TCA TCT TGA AATGATTCTCATTGATTAATTTGCCATAATAACACTTGCACATGTGACTCTGGTCAATTCAAAA
        * *   * ser   * asp arg asp leu gly ser ser OP    **      *     * *  ***   ****  ****  ***********  *******   **

GACTCTCACCCCCTGCTATAACTATGACCATGCTGATAAACTGA
GACTCTTATTTCGGCTTTAATCACAGAATTGACTGAATTAGTTCTGCAAATACTTTGTCGGTATATTAAGCCAGTATATGTTAAAAAGACTTAGGTTCAGGGGCATCAGTCCCTAAGATG
      * *** *   *   ** ******  ***** **********************************************************************************

    TTTATCTATTTAAATATTTATT TAACTATTCATAAGATTTAAATTATTTTTGTTCATATAACGTCA TGTGCACCTTTACACTGTGGTTAGTGTAATAAAACATGTTCCTTATA
TTATTTATTTTTACTCATTTATTTATTCTTACATTTTATCATATTTATACTATTTATATTCTTATATAACAAATGTTTGCCTTTACATTGTATTAAGA TAACAAAACATGTTCAGCTTT
***** * * *  * * **        * *  **  * * *     * *     * *  *     *****  *  ***          *  ** *  **  *-            **** *

TTTACTCAATCCATTATTTTGTGTTG    TTCATTAAACTTTTACTATAGGAACTTCCTGTATGTGTTTCATTCTTTAATATGAAATTCCTAGCCTGACTGTGCAACCTGATTAGAGAAT
CC ATTTGGTTAAATATTGTATTTTGTTATTTATTAAATTATTTTCAAACAAAACTTCTTGAAGTTATTTATTCGAAAACCAAAATCCAAACACTAGTTTTCTGAACCAAATCAAGGAAT
*** * *** ** *    * * *   ***  *_____* *  *** * **  ** *  ** *  **  *  ****  ****  ******** ** ** ****    **  * **
                                        Δ

AAAGGGTATATTTTATTTGCTTATCATTATTATATGTAAGA
GGACGGTAATATACACTTACCTATTCATTCATTCCATTTAC
** *    *** ** *  *  *  *** ***** *** ****
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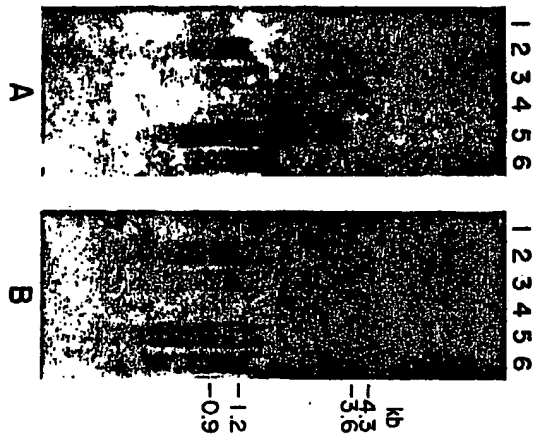

Fig.7.